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FOREWORD

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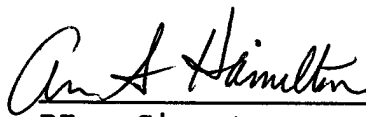
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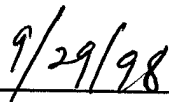
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A. INTRODUCTION

The purposes of the study are the following:

- (1) To determine the effect of moderate exercise on estradiol (and progesterone levels) over an entire menstrual cycle by means of daily salivary samples in healthy premenopausal identical twins who differ in the amount of physical exercise activity per week.
- (2) To determine the effect of moderate exercise on menstrual cycle length, (specifically luteal phase length among identical twins).

Overview: Exercise has been shown to be associated with a reduced risk of breast cancer. There is evidence that women exercising, for an hour or more per day, have lower serum estrogen (estradiol) levels than sedentary women (due to more anovular cycles and lower estrogen levels in ovular cycles). These lower estrogen levels appear most likely to be the mechanism behind their reduced breast cancer risk, however much is still unknown. Previous studies have, for the most part, focused on the effects of high exercise levels among athletes, as opposed to more moderate levels of exercise, on estrogen levels, and they may have been subject to 'selection bias', i.e. women who exercise may do so because of predisposing hormonal factors. In addition, the estradiol measurements have usually been based on only a few serum samples taken at different times during a menstrual cycle. This study is addressing these issues by using 60 sets of monozygous twins who are discordant with regard to moderate exercise habits (i.e. sedentary vs. exercising an average of 20 minutes/day), but are identical for heritable aspects of body build and constitution. Estradiol is being measured on a daily basis by use of salivary samples collected during a complete menstrual cycle. The subjects are being selected from 426 pairs of healthy premenopausal identical twins under the age of 45 who participated in the California Twin Cohort Study. They are being screened to determine eligibility (i.e. neither twin having an endocrine or metabolic disorder and the pair discordant for current amount of physical exercise activity), before being asked to participate. The use of the salivary samples is an innovative method for the measurement of estradiol and offers distinct advantages over the more traditional serum hormone measurements for which daily samples are not practical. Repeated sampling, as compared to single or infrequent sampling of individuals makes it possible to more accurately characterize ovarian function and allows for a more complete assessment of estradiol levels over different phases of the menstrual cycle, without the discomfort of venipuncture or the inconvenience of office visits. Salivary steroids have been shown to be extremely stable when samples are properly treated and this method of collection is ideally suited for use in the proposed study where subjects are located throughout California. The hormone assays are being done by Dr. Peter Ellison (Co-Investigator), an expert in the analysis of and validation of salivary samples. We are also obtaining information on daily physical exercise activity during the month of sample collection and dietary intake using established and well tested questionnaires. Analysis of covariance methods will be used to assess the relationship of estrogen levels during different parts of the menstrual cycle to exercise, controlling for diet, body mass, and other potentially confounding factors. Based on the sample size of 60 pairs of twins, we have the power to detect differences in estradiol levels of 15% between the sedentary and moderately exercising twins. The study has important public health implications in developing strategies for the prevention of breast cancer.

Background: Hormones and breast cancer risk: A great deal of evidence exists suggesting that ovarian hormones, in particular estrogens, play a major role in breast cancer risk [1-3]. The relationship between age and breast cancer incidence supports this theory. The age-incidence relationship of the common non-hormone related cancers such as colon, stomach and bladder show a continuous steady increase with age. In contrast, breast cancer incidence increases steadily and rapidly with age until about age 50 (average age at menopause) at which time the rate of increase slows dramatically [2]. Direct epidemiological study of the effect of age at menopause shows that for each year a woman's ovaries continue to function there is a 10% increase in her subsequent breast cancer risk [1,2,6]; this is true whether the menopause is natural or artificial (bilateral oophorectomy). The decline in the rate of increase in population incidence around age 50, is thus directly correlated with the markedly reduced serum levels of estrogen (and progesterone) after menopause. Further evidence is provided by the epidemiological observation that women who begin menstruating early have a higher risk of breast cancer than women whose menarche is delayed [1].

Initial reports on estrogen levels in premenopausal breast cancer cases and controls have been inconclusive; however, our recent studies, which paid strict attention to factors which may influence hormone levels in cases, found statistically significant elevated serum levels of estradiol in premenopausal breast cancer cases compared to closely matched controls [7]. The increased levels of estrogen presumably increase risk through their known action as a breast cell mitogen [2]. Luteal phase estradiol (E2) is of major concern, but follicular E2 is also of interest as the breast cell proliferation rate in the follicular phase is some 50% that in the luteal phase and so is a significant contributor to total breast cell proliferation [2]. There is evidence, though controversial, that progesterone also acts to increase breast cell proliferation [2].

Exercise and breast cancer risk: We recently completed a large case-control study of breast cancer cases and healthy controls [4]. Compared to inactive women, risk of breast cancer was reduced by 27% in women who exercised on average 2 ½ hours per week, and was reduced by 58% in women who exercised 4 or more hours per week (average approximately 60 mins/day), after controlling for established breast cancer risk factors. Our study supports two previous investigations of exercise and breast cancer risk [5,8], and has been confirmed by two subsequent studies [10,11] which also showed a positive association between exercise and lower risk of breast cancer. However, the study based on the NHANES I did not support these findings[9].

Exercise and ovarian function: We believe, based on our understanding of the relation of ovarian hormones to breast cancer risk [2,3], that the observed protective effect of exercise against breast cancer is likely to be due to a reduction in exposure to serum estrogens. Reduced serum estrogen levels may be due to an increased frequency of anovulatory cycles and/or to decreased circulating levels of estrogen in ovulatory cycles. More exposure to estrogen occurs during the luteal phase of the cycle; thus length of this portion of the cycle as well as overall cycle length may also affect level of estrogen exposure.

The prevalence of oligomenorrhea and amenorrhea is higher among athletes than among the general population [12-14]. Studies conducted among athletes and sedentary controls show a consistent association between physical activity and increased frequency of anovulation [15-19]. Only two studies exist which provide data on the effect of moderate exercise activity on the frequency of anovulation. One of these studies is based on only 6 women joggers [16], while the other only included adolescents [20]. These studies also found a higher incidence of anovulation among the more physically active women.

The literature regarding the association of extent of exercise activities and basal levels of ovarian hormones in ovulatory cycles is scant. The only studies with interpretable data are the two studies [18, 21] which confirmed ovulatory status on the basis of an objective serum progesterone measurement. These studies showed that women who exercise have lower levels of circulating estradiol than sedentary controls.

Cycles with long follicular phases are associated with lower than normal cumulative E2 exposure since such cycles have an increased number of days with early follicular phase low E2 levels. Cycles with short luteal phases have also been found to be associated with low E2 values [22].

It has not been established whether cycle length of ovulatory cycles, and in particular luteal phase length, is affected by exercise activity. Three studies [23-25] reported shorter luteal phase lengths in athletes, with no consistent effect on follicular length, but their luteal phase results were not confirmed by other studies [18].

Salivary steroids and ovarian function: Clinical and research applications of salivary steroid assays have spread steadily since their introduction in the late 1970's [26-30]. Salivary steroid levels are considered to reflect biologically active free steroid levels, and so may be functionally more specific than either plasma or urinary steroid determinations. Salivary progesterone measurements have proven particularly useful in the characterization of ovarian function, particularly due to the ease of collecting repeated samples from individual subjects in the field [28, 31-35]. Salivary progesterone profiles have been found to correspond closely to urine and plasma profiles [36], and to coordinate temporally with the timing of the LH surge [37] and the rupture of the dominant follicle [38, 39]. Standards of salivary progesterone levels have been established for both clinical [37, 40-42] and epidemiological [39, 43] applications.

Highly sensitive assays have also been developed for the detection of estradiol in the saliva [27, 43-45]. Salivary estradiol profiles demonstrate a close temporal relationship to salivary progesterone profiles and are highly correlated with plasma levels [45, 46].

B. BODY

Summary of work done during the first year:

- 1) Developed screening questionnaire and general questionnaire
- 2) Assembled the Study Kit which includes the introductory letter, informed consents, the saliva collection tubes and associated materials (including instructions, gum, pen, log sheet), questionnaires (general, diet, and daily activity logs), and return mailing supplies into a box for mailing to each participant.
- 3) Identified identical premenopausal female twins discordant for exercise from the California Twin Program.
- 4) Conducted telephone interviews with selected twins ask screening questions to determine eligibility.
- 5) Mailed kits to eligible pairs and received completed questionnaires at USC and saliva samples at Harvard University.

The above tasks are consistent with Technical Objectives 1-4 as listed below:

Technical objectives 1-4: Selection of twins and collection of saliva samples: Ongoing throughout Yrs. 1, 2, and during the first 6 months of Year 3.

1. During the course of the study identical female twins will be selected who previously participated in the California Twin Cohort and indicated that they are premenopausal .
2. These pairs will called on the telephone and re-interviewed regarding factors related to their eligibility.
3. Once a pair is determined to be eligible and they agree to participate they will be mailed informed consent forms, saliva sample collection kits, and exercise and dietary questionnaires.
4. We will check with them periodically to determine when the first day of their period occurs and assure that they are following the directions for collection of the saliva samples.
5. They will mail their completed sample kits to Dr. Ellison's laboratory and the completed questionnaires to USC.

With regard to Technical Objective 5, the completed kits have been sent to Harvard and the first batch of hormonal assays will be completed in the near future.

Technical Objective 5: Completion of Hormonal Assays: Year 1, month 3 through Year 3, Month 9.

1. Dr.Ellison's Laboratory will receive the kits and will be blinded as to which twin is performing more exercise.
2. The laboratory assistant will complete the hormonal assays according to standard protocols.
3. Results will be sent to USC.

No work has yet been done on Technical Objectives 6-7, but data entry and dietary analyses of the first batch of questionnaires will be done shortly.

Technical Objectives 6-7: Data Management: Year 1, Month 6-Year 3 Month 10

1. Physical Activity questionnaires will be coded and entered at USC.
2. Dietary questionnaires will be sent to Dr. Willett for analysis, with results being sent to USC.

3. Hormonal assay data will be merged with the questionnaire data.

Work has not yet begun on Technical Objectives 8-9.

Technical Objectives 8-9: Data analysis and publishing of papers: Year 1, Month 12-Year 3, Month 12.

1. Preliminary and final analyses will be performed to address the stated hypotheses.
2. Papers will be published on the results.

Specific Description of Work Completed:

Questionnaire Development

Screening Questionnaire: The screening questionnaire (Appendix 1) was designed to determine if both twins currently meet the eligibility requirements for the study which include:

- 1) both twins are still premenopausal and have had regular periods in the last 6 months,
- 2) both twins were parous or both twins were nulliparous.
- 3) neither have had breast, ovarian, uterine, or cervical cancer,
- 4) neither have been diagnosed with an endocrine or metabolic disorder,
- 5) neither have taken oral contraceptives, hormone replacement therapy, or steroid medications of any type in the past 6 months,
- 6) neither have been pregnant or lactated within the past year, and
- 7) they differ in their exercise levels

This screening questionnaire is administered over the telephone. Once both twins have been interviewed a determination is made regarding the pair's eligibility for the study based on both twins' responses. If eligible, both members are called back and asked to participate, and if they agree, they are then sent the Study Kit.

General Questionnaire: The general questionnaire was finalized and is included in the Study Kit (see below) (Appendix 2). It obtains information on physical activity, current weight, height, reproductive history, use of infertility drugs and oral contraceptives, family history of cancer, smoking and alcohol use, and background information. The participants fill out the questionnaire and mail it back to us along with other study materials.

Contents of Study Kit:

The following items are included in the Study Kit and mailed in a box by priority mail (at a cost of \$4) to each participant:

- 1) Introductory Letter (Appendix 3)
- 2) Informed Consent (Appendix 4)
- 3) Saliva Donation form (Appendix 5)
- 4) General Questionnaire (Appendix 2)
- 5) 40 copies of Physical Activity Daily Log (Appendix 6)
- 6) Willett Diet Assessment form (and #2 pencil) (Appendix 7)
- 7) Saliva collection materials (supplied by Dr. Ellison's laboratory at Harvard) including:

- a. 40 saliva collection tubes (with ID numbers) in plastic container
 - b. Sharpie pen for writing dates, initials, and time of collection on tubes
 - c. Carefree chewing gum to assist with saliva flow
 - d. Collection instructions (Appendix 8)
 - e. Saliva collection log sheet (Appendix 9)
- 8) Pre-addressed and pre-stamped (\$3 priority mail) brown return envelope for mailing of signed Informed consent, saliva donation form, general questionnaire, diet assessment, and physical activity logs to USC.
- 9) Pre-addressed and pre-stamped (\$3 priority mail) cardboard return box for mailing of the saliva collection tubes and saliva collection log to Harvard.

Identification of Female Identical Twins for Screening and Screening Results

Initially 180 pairs of identical female twins from the California Twin Program were selected for screening based on the following information derived from the questionnaire they completed in 1992:

- a. they were both premenopausal (and had not had a hysterectomy) and under 45 in 1992.
- b. neither reported having had breast, ovarian, uterine, or cervical cancer, diabetes, asthma, or Graves disease. In addition, neither were on kidney dialysis, or taking insulin, oral diabetes medicine, or prednisone or other steroids.
- c. their total minutes of exercise reported per week differed by at least 20 minutes and one of the twins was sedentary.

We did not exclude twins if they said they had taken oral contraceptives in 1992 at the time of the original questionnaire, because we wanted to ask them about their current use of these drugs during the screening interview since their use may have changed during the interim.

Results of Screening interviews

To date, both twins from 135 of the 180 originally identified pairs have been interviewed over the telephone to determine their eligibility. The results are shown in Table 1.

At this time, 16 eligible pairs are participating in the study. Reasons given by those 4 pairs of twins who were eligible, but one member refused to participate, mainly included being too busy. Since we are asking that the individual participant collect a saliva sample every day for approximately 30 days and complete questionnaires, it is understandable that some may not wish to participate. However, of those who have said yes, we have had no drop outs.

We are still making an effort to locate the lost pairs and are continuing to screen the remaining 45 pairs originally identified. An additional 246 pairs are available to contact from the California Twin Program; however, they did not differ in exercise level in 1992. Since changes may have occurred in the interim, they may be eligible at this time. A more promising source of eligible pairs is from a new phase of the California Twin Program. In June, new questionnaires were mailed to approximately 35,000 twins who are in the age group 10 years younger than the original group. We are confident that we will lose fewer pairs to menopause in this new group and their exercise and hormone use information will be current; hence eligible pairs will be more likely to be identified directly from the questionnaire and less time will need to be spent screening pairs.

These questionnaires are in the process of being scanned and data from them will be available before the end of the year. Thus we feel confident that we be able to identify enough additional eligible pairs to complete the original sample size requirements of 60 pairs over the 3 year grant period.

Table 1: Results of Screening Interviews

Result of Screening of Both Members of Pair	Number of Pairs	Percent of Total Pairs Screened
Eligible and consented	16	10.5
Eligible and refused	4	3.0
Screened and not eligible because:	93	68.9
1+ had menopause	(12)	(9.0)
1+ had very irregular periods	(2)	(1.5)
Parity discordant	(19)	(14.3)
1 + had disqualifying disease	(5)	(3.8)
1+ taking OC's or hormones	(31)	(23.3)
1+ taking cortisone/prednisone	(5)	(3.8)
1+ breast fed a child or pregnant within past year	(4)	(3.0)
Multiple of above reasons	(8)	(6.0)
Both had same exercise level	(7)	(5.3)
Lost, could not screen	15	11.3
Refused screening interview	7	5.3
Total	135	100.0

Completion of Study Requirements by Eligible Pairs

Study Kits have been mailed to the 16 eligible pairs (32 individuals) who agreed to participate. To date, we have received completed questionnaires and saliva collection tubes back from both members of 10 of these pairs. The remaining 6 pairs are currently in the process of collecting the samples as they were mailed the Kits within the past 2 months. One pair decided that it would be too much to do after receiving the Kit, but upon further conversation with them, it was determined that they didn't object to collecting the saliva samples, but didn't want to complete all of the accompanying questionnaires. We waived the daily physical activity log, and eliminated some of the questions in the general questionnaire, and then they were willing to participate.

Laboratory Procedures

The samples from the 10 pairs have been received at Harvard and they are currently evaluating the samples prior to performing the assays. A saliva collection log form was added after about half of the Kits had been mailed, and we are currently calling some twins to resolve some ambiguities about the days of menstrual flow. One twin missed collecting saliva on several days during her

cycle. It might be necessary to ask her to repeat the collection. Otherwise, the samples appear to have been collected according to the instructions given.

C. CONCLUSIONS

The first year of the study has included development of study procedures, questionnaires, and coordination of work with Dr. Ellison's Laboratory. Screening interviews have been conducted with 135 pairs. In general, the eligible twins who agreed to participate (16 pairs so far) are willing to complete the rather demanding requirements of the study; however we had fewer pairs that were eligible than anticipated. Also, we found that a saliva collection log form needed to be added to the Study Kit and this procedure has been implemented. Additional twins will soon be available for screening and participation due to the implementation of the second phase of the California Twin Program (funded by other sources). Thus, these initial problems are not seen as problematic. In the next year, we will continue to screen and enroll twins for participation, data entry of questionnaires will be initiated, a batch of completed Willett dietary assessments will be sent for analysis, and we expect to have the laboratory hormonal assays completed on all pairs who have participated. Procedures will be developed for merging the data from these various sources into a unified record.

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E. APPENDICES

1. Screening Questionnaire
2. General Questionnaire
3. Letter to participants
4. Informed consent
5. Saliva Donation Form
6. Physical activity daily log sheet
7. Diet Questionnaire
8. Saliva collection instructions
9. Saliva collection log form

**CALIFORNIA TWIN PROGRAM EXERCISE STUDY
SCREENING QUESTIONNAIRE**
Answers for Twin 1 (Seqno=1) Answers for Twin 2 (Seqno=2)

Name: _____

Date: _____

1) First of all, to verify our information, what is your birthdate?

Month/Day/Year Month/Day/Year

2) Have you ever been pregnant?

____ 1. No (*Skip to Q.3*)
____ 2. Yes

____ 1. No (*Skip to Q.3*)
____ 2. Yes

If yes: **A. How many live born children have you had?**

Number _____ Number _____

B. Have you been pregnant at any time during the past year?

____ 1. No (*Skip to Q.3*)
____ 2. Yes: **What was the
outcome?**

____ 1. No (*Skip to Q. 3*)
____ 2. Yes: **What was the
outcome?**

____ 1. Livebirth
____ 2. Miscarriage/Abortion
____ 3. Stillbirth
____ 4. Other: _____

____ 1. Livebirth
____ 2. Miscarriage/Abortion
____ 3. Stillbirth
____ 4. Other: _____

What was the date
that your baby was
born (or pregnancy
ended)?

m/d/y

What was the date
that your baby was
born (or pregnancy
ended?)

m/d/y

3) Have you breast fed a baby in the past year?

- ___ 1. No
___ 2. Yes: When did you
stop?

- ___ 1. No
___ 2. Yes: When did you
stop?

m/d/y

m/d/y

4) During the past year, have you taken any of the following hormone containing
preparations?

A. Oral contraceptives, (including birth control pills, depo-provera shots, or
norplant implants)?

- ___ 1. No
___ 2. Yes: What did you take?

- ___ 1. No
___ 2. Yes: What did you take?

(If unsure ask: Do you have the bottle or pill container?
Can you get the information and I will call you back?)

In what month did
you last take them ?

In what month did
you last take them?

Month: _____

Month: _____

B. Hormone Replacement Therapy pills such as Premarin, or the patch?

- ___ 1. No
___ 2. Yes: What did you take?

- ___ 1. No
___ 2. Yes: What did you take?

(If unsure ask: Do you have the bottle or pill container?
Can you get the information and I will call you back?)

In what month did
you last take them?

In what month did
you last take them?

Month: _____

Month: _____

C. Any other steroid drugs such as prednisone or cortisone, sometimes prescribed for acne, asthma, or inflammation?

- ☐ 1. No
☐ 2. Yes: What did you take?

- ☐ 1. No
☐ 2. Yes: What did you take?

(If unsure ask: Do you have the bottle or pill container?
Can you get the information and I will call you back?)

In what month did
you last take them?

In what month did
you last take them?

Month: _____

Month: _____

5) Have you had a menstrual period in the last 6 months?

- ☐ 1. No
☐ 2. Yes (Skip to Q. 6)

- ☐ 1. No
☐ 2. Yes (Skip to Q. 6)

If no: What was the month and year when
you had your last period?

_____/_____
mo. yr.

Why did your periods stop?

- ☐ 1. Natural menopause
☐ 2. Surgery (hysterectomy)
☐ 3. Other: _____

(Skip to Q. 14)

If no: What was the month and year when
you had your last period?

_____/_____
mo. yr.

Why did your periods stop?

- ☐ 1. Natural menopause
☐ 2. Surgery (hysterectomy)
☐ 3. Other: _____

(Skip to Q. 14)

6) When did your last menstrual period start?

month/day/year

month/day/year

7) Overall, would you say that your periods are regular? (that is, you can usually predict about when the next period will start)?

- ☐ 1. No (Skip to Q. 9)
☐ 2. Yes

- ☐ 1. No (Skip to Q. 9)
☐ 2. Yes

8) About how many days do you usually have between the first day of one menstrual period (i.e. the first day of bleeding) and the first day of the next period? Would you say:

- ☐ 1. Less than 20
- ☐ 2. 20-24
- ☐ 3. 25-29
- ☐ 4. 30-34
- ☐ 5. 35+
- ☐ 6. Can't predict

- ☐ 1. Less than 20
- ☐ 2. 20-24
- ☐ 3. 25-29
- ☐ 4. 30-34
- ☐ 5. 35+
- ☐ 6. Can't predict

9) How many periods have you had in the last 6 months?

Number _____

Number _____

10) Have you experienced hot flashes in the past year?

- ☐ 1. No
- ☐ 2. Yes

- ☐ 1. No
- ☐ 2. Yes

If yes: When did you first experience them?

_____/_____
mo. yr.

If yes: When did you first experience them?

_____/_____
mo. yr.

About how often do you have them?

Number _____ per _____
(day, week, month)

About how often do you have them?

Number _____ per _____
(day, week month)

11) In the past year, did you participate in any regular exercise such as jogging, swimming, aerobics, bicycling, tennis, weight-lifting, or other activities that make a person breathe hard?

- ☐ 1. No (Skip to Q. 12)
- ☐ 2. Yes

- ☐ 1. No (Skip to Q. 12)
- ☐ 2. Yes

If yes: A. In what activities do you (did you) participate?

B. When did you start?

_____/_____
mo. yr.

_____/_____
mo. yr.

C. Are you still exercising now?

___ 1. No: When did you stop?

___ 1. No: When did you stop?

___ 2. Yes
_____ month

___ 2. Yes
_____ month

D. How many times per week do you (did you) usually participate in regular exercise?

Number _____

Number _____

(If can't give an answer, prompt with the following intervals) Would you say::

- ___ 1. Once
- ___ 2. 2-4
- ___ 3. 5-7
- ___ 4. 8-10
- ___ 5. 11 or more

- ___ 1. Once
- ___ 2. 2-4
- ___ 3. 5-7
- ___ 4. 8-10
- ___ 5. 11 or more

E. When you exercise(d), about how many minutes, on average, do (did) you perform the exercise each time?

Number of minutes _____

Number of minutes _____

(If can't give an answer, prompt with the following intervals) Would you say:

- ___ 1. Under 15 minutes
- ___ 2. 15-29 minutes
- ___ 3. 30-59 minutes
- ___ 4. 60 minutes or more

- ___ 1. Under 15 minutes
- ___ 2. 15-29 minutes
- ___ 3. 30-59 minutes
- ___ 4. 60 minutes or more

12) In the past year, did you participate in any other regular exercise such walking, golfing, gardening, and other forms of exercise that usually do not make a person breathe hard?

___ 1. No (Skip to Q. 13)

___ 1. No (Skip to Q. 13)

___ 2. Yes

___ 2. Yes

If yes: A. In what activities do you (did you) participate?

B. Are you still doing this type of exercising now?

___ 1. No: When did you stop?

___ 1. No: When did you stop?

___ 2. Yes
_____ month

___ 2. Yes
_____ month

C. How many times per week do you (did you) usually participate in regular exercise?

Number _____

Number _____

(If can't give an answer, prompt with the following intervals) Would you say::

- ___ 1. Once
- ___ 2. 2-4
- ___ 3. 5-7
- ___ 4. 8-10
- ___ 5. 11 or more

- ___ 1. Once
- ___ 2. 2-4
- ___ 3. 5-7
- ___ 4. 8-10
- ___ 5. 11 or more

D. When you exercise(d), about how many minutes, on average, do (did) you perform the exercise each time?

Number of minutes _____

Number of minutes _____

(If can't give an answer, prompt with the following intervals) Would you say:

- ___ 1. Under 15 minutes
- ___ 2. 15-29 minutes
- ___ 3. 30-59 minutes
- ___ 4. 60 minutes or more

- ___ 1. Under 15 minutes
- ___ 2. 15-29 minutes
- ___ 3. 30-59 minutes
- ___ 4. 60 minutes or more

13) Now I am going to read a short list of medical conditions. Please stop me if a doctor has ever told you that you had a condition I have mentioned. Has a doctor ever told you that you had :

Ovarian cysts ___ 1. No ___ 2. Yes
Polycystic ovaries, or ___ 1. No ___ 2. Yes
Stein Levanthal Syndrome

___ 1. No ___ 2. Yes
___ 1. No ___ 2. Yes

Diabetes, Sugar diabetes ___ 1. No ___ 2. Yes
or high blood sugar

___ 1. No ___ 2. Yes

Glandular conditions ___ 1. No ___ 2. Yes
of the pituitary or
adrenal glands

___ 1. No ___ 2. Yes

Any of the following thyroid diseases:

Grave's Disease ___ 1. No ___ 2. Yes

___ 1. No ___ 2. Yes

Hashimoto's disease
or chronic thyroiditis

___ 1. No ___ 2. Yes

___ 1. No ___ 2. Yes

Overactive thyroid
(hyperactive)

___ 1. No ___ 2. Yes

___ 1. No ___ 2. Yes

Underactive thyroid
(hypoactive)

___ 1. No ___ 2. Yes

___ 1. No ___ 2. Yes

Any type of Cancer?

___ 1. No ___ 2. Yes

___ 1. No ___ 2. Yes

What type (site)?

How old were you:
when first diagnosed?

14) Have you ever had a mammogram?

___ 1. No
___ 2. Yes

___ 1. No
___ 2. Yes

When was the last time?

_____/_____
mo. yr.

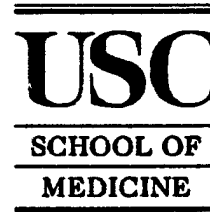
When was the last time?

_____/_____
mo. yr.

Those are all the questions I have for you. Thank you very much for your time and effort.
We will be reviewing your answers as well as those of your twin and will be calling you
back to let you know if you and your twin are eligible.

APPENDIX 2
General Questionnaire

USC/Norris Comprehensive Cancer Center
University of Southern California
Department of Preventive Medicine
1441 Eastlake Ave., Mail Stop 44
Los Angeles, California 90033-0800



Name: _____

Study Number: _____

**An Innovative Assessment of Endogenous Estrogen Activity
in Persons with Different Habits of Exercise**

General Questionnaire

Any Questions?

Please call and leave a message at our Toll Free Number (1-800-421-9631), or you may call the Principal Investigators directly: Dr. Ann Hamilton at 213-764-0434 (after June 13 the number will be 323-865-0434), or Dr. Lisa Shames at 213-764-0422 (after June 13, 323-865-0422). Our FAX number is 213-764-0141 (323-865-0141 after June 13). Dr. Hamilton can also be reached by e-mail at ahamilt@hsc.usc.edu.

STUDY NUMBER _____

Please fill in today's date: _____ / _____ / _____
Month Day Year

I. Physical Activity Profile:

1. Please list any sport or recreation you have actively participated in during the past YEAR and how frequently you have participated in it, as indicated in the table.
Please remember to include seasonal sports or events.

NAME OF SPORT, RECREATION OR OTHER PHYSICAL ACTIVITY	Number of WEEKS per YEAR you participate in the activity	Average TIME spent per WEEK doing the activity when active HOURS / MINUTES	Total Number of YEARS you have participated
1.		/	
2.		/	
3.		/	
4.		/	
5.		/	

2. Please list any sport or recreation you have actively participated in during the past WEEK.
Please remember to include seasonal sports or events.

NAME OF SPORT, RECREATION OR OTHER PHYSICAL ACTIVITY	Number of TIMES you have participated in the activity this WEEK	Average TIME SPENT per episode HOURS / MINUTES
1.		/
2.		/
3.		/
4.		/
5.		/

3. Approximately how many flights (not numbers) of stairs do you usually climb up each day?

_____ Flights

4. Approximately how many city blocks or their equivalent do you regularly walk each day?

_____ Blocks

5. On a usual weekday and a weekend day, how much time do you spend on the following activities? Total for each day should add up to 24 hours.

TYPE OF ACTIVITY	Usual Week Day HOURS PER DAY	Usual Weekend Day HOURS PER DAY
a. Vigorous activity (e.g., digging in the garden, strenuous sports, jogging, chopping wood, sustained swimming, brisk walking, heavy carpentry, bicycling on hills)		
b. Moderate activity (e.g., housework, light sports, regular walking, golf, yard work, lawn mowing, painting, repairing, light carpentry, dancing, bicycling on level ground)		
c. Light activity (e.g., office work, driving a car, strolling, personal care, standing with little motion)		
d. Sitting activity (eating, reading, desk work, watching TV, listening to radio)		
e. Sleeping or reclining		
	Total=24 hours	Total=24 hours

6. At least once a week, do you engage in regular activity akin to brisk walking, jogging, bicycling, etc. long enough to work up a sweat, get your heart thumping, or get out of breath?

____ 1) Yes
____ 2) No

7. What is your usual pace of walking?

(Please check one)

____ 1) Casual or strolling (<2 mph)
____ 2) Average or normal (2 to <3 mph)
____ 3) Fairly brisk (3 to <4 mph)
____ 4) Brisk or striding (4 mph or faster)

8. Do you have a job (either full or part-time)?

____ 1) Yes, full time
____ 2) Yes, part time
____ 3) No

- 8.1 *If Yes*, How much of the time on your job do you usually walk, climb stairs, lift heavy items, or do other physical activities (other than sitting or standing)?

(Please check one)

____ 1) None of the time
____ 2) Less than half the time
____ 3) At least half the time
____ 4) Most of the time
____ 5) All the time

9. Have you ever (including childhood) been a member of a competitive team (where you attended practice sessions, workouts, training sessions or competitions more than two times a week)? This would include intramural, varsity or amateur athletic union sports (e.g., volleyball, swimming, gymnastics, tennis), serious participation in ballet, dance or exercise classes on a regular basis and regular jogging/running of a least one mile at each workout.

____ 1) Yes
 ____ 2) No

9.1 *If Yes*, In the table below,

- A. Please list the activities you participated in,
 B. The age when you first began participating,
 C. The age when you stopped,
 D. The total number of years your participated in the activity during these ages
 E. On average, how many hours per week you participated in the activity, when you were active?

A. Name of Activity	B. Age began	C. Age stopped	D. Total Years	E. Hours per week
1.				
2.				
3.				
4.				
5.				

10. How tall are you without shoes?

____ Feet ____ Inches

11. a) How much do you currently weigh (in pounds)?

____ Pounds (Current)

- b) How much did you weigh at the age of 20 (in pounds)?

____ Pounds (Age 20)

12. a) What has been the most you have weighed?
 (Not including times when you were pregnant)

____ Highest Weight

- b) What has the least you have ever weighed (as an adult)?

____ Lowest Weight

13. How many times have you lost or gained 25 pounds or more?

____ 1) Never
 ____ 2) 1 time
 ____ 3) 2-3 times
 ____ 4) 4+ times

14. Compared to this time a year ago, how much has your weight changed?

- _____ 1) No change
- _____ 2) Gained 5 lbs.
- _____ 3) Gained 6-10 lbs.
- _____ 4) Gained 11+ lbs.
- _____ 5) Lost 5 lbs.
- _____ 6) Lost 6-10 lbs.
- _____ 7) Lost 11+ lbs.

15. How does your hip measurement compare to your waist measurement?

- _____ 1) Hips much bigger
- _____ 2) Hips bigger
- _____ 3) About the same
- _____ 4) Waist bigger
- _____ 5) Waist much bigger

II. Reproductive History

13. What was your age at your first menstrual period?

_____ Age

14. How long did it take, after your periods began, for them to become regular?
(That is, you could predict within a day or two when your next period would occur)

(Please check one)

- _____ 1) Less than 2 months
- _____ 2) From 2-6 months
- _____ 3) From 7-12 months
- _____ 4) More than a year
- _____ 5) Never regular

15. How many times have you been pregnant?

_____ Number

16. How old were you when you had your first pregnancy?

_____ Age

17. What was the outcome of your first pregnancy?

- _____ 1) Live born child
- _____ 2) Miscarriage/Abortion
- _____ 3) Stillbirth
- _____ 4) Other

18. How many live born children have you had?

_____ Number

19. How many boys? How many girls?

_____ Number of boys

_____ Number of girls

STUDY NUMBER _____

20. How old were you when you had your first live born child?

_____ Age

21. How old were you when you had your last live born child?

_____ Age

22. Have you given birth to twins?

_____ 1) Yes
_____ 2) No

23. Totaling all periods of breast feeding and all children, how many months in total would you estimate that you have breast fed?

_____ 1) Never breast fed
_____ 2) 1-5 months
_____ 3) 6-11 months
_____ 4) 12-23 months
_____ 5) 24-35 monts
_____ 6) 36 or more months

24. Did you ever consult a physician about a fertility problem?

_____ 1) Yes
_____ 2) No

If Yes: What was the cause of the problem?

_____ 1) No problem found
_____ 2) A problem with you
_____ 3) A problem with your husband
_____ 4) A problem with both of you
_____ 5) Never resolved

25. Did you ever get treatment for an infertility problem?

_____ 1) No
_____ 2) Yes, took fertility drugs
_____ 3) Yes, had in vitro fertilization
_____ 4) Yes, other treatment

26. Have you ever taken oral contraceptives (birth control pills?)

_____ 1) Yes
_____ 2) No

If Yes:

26.1 At what age did you first take them?
Last take them?

_____ Age First

_____ Age Last

26.2 For how many years altogether have you taken them?

_____ Total Years

III. Family History

27. For each of your relatives listed in the chart below, please fill in the information indicating:

A. If they are still living, and if so their current age

If not living, their age at death

B. If they have ever had cancer, and

C. If so, how old they were when it was diagnosed, and where in the body the cancer started.

These questions pertain to your biological relatives only, please do not include adopted, or step relatives. If you don't have sisters (other than your twin) or brothers, leave those spaces blank.

Relative	A. Still Living?	B. Ever had cancer?	C. If Yes
1. Mother	___ Yes: Current age ____ ___ No: Age at death: ____	___ Yes ___ No:	Age diagnosed: ____ Cancer site: ____
2. Father	___ Yes: Current age ____ ___ No: Age at death: ____	___ Yes ___ No:	Age diagnosed: ____ Cancer site: ____
3. Sister #1 (other than twin)	___ Yes: Current age ____ ___ No: Age at death: ____	___ Yes ___ No:	Age diagnosed: ____ Cancer site: ____
4. Sister #2 (other than twin)	___ Yes: Current age ____ ___ No: Age at death: ____	___ Yes ___ No:	Age diagnosed: ____ Cancer site: ____
5. Brother #1	___ Yes: Current age ____ ___ No: Age at death: ____	___ Yes ___ No:	Age diagnosed: ____ Cancer site: ____
6. Brother #2	___ Yes: Current age ____ ___ No: Age at death: ____	___ Yes ___ No:	Age diagnosed: ____ Cancer site: ____
(If additional brothers/sisters list below)	___ Yes: Current age ____ ___ No: Age at death: ____	___ Yes ___ No:	Age diagnosed: ____ Cancer site: ____
7. _____	___ Yes: Current age ____ ___ No: Age at death: ____	___ Yes ___ No:	Age diagnosed: ____ Cancer site: ____
8. _____	___ Yes: Current age ____ ___ No: Age at death: ____	___ Yes ___ No:	Age diagnosed: ____ Cancer site: ____
9. _____	___ Yes: Current age ____ ___ No: Age at death: ____	___ Yes ___ No:	Age diagnosed: ____ Cancer site: ____

28. Did either of your grandmothers have cancer ?

- a. Maternal Grandmother ___ 1) Yes: Cancer Site: _____
 ___ 2) No
 ___ 3) Don't Know

- b. Paternal Grandmother _____ 1) Yes: Cancer Site: _____
 _____ 2) No
 _____ 3) Don't Know

29. Did any of your aunts (mother's sisters or father's sisters) have cancer?

- a. Mother's Sisters** _____ 1) Yes: Cancer Site(s): _____
 _____ 2) No
 _____ 3) Don't Know
 _____ 4) Mother had no sisters

- b. Father's Sisters**
- _____ 1) Yes: Cancer Site(s): _____
- _____ 2) No
- _____ 3) Don't Know
- _____ 4) Father had no sisters

30. Did any of your uncles (mother's brothers or father's brothers) have cancer?

- a. Mother's Brothers
- _____ 1) Yes: Cancer Site(s): _____
- _____ 2) No
- _____ 3) Don't Know
- _____ 4) Mother had no brothers

- b. Father's Brothers**
- _____ 1) Yes: Cancer Site(s): _____
- _____ 2) No
- _____ 3) Don't Know
- _____ 4) Father had no brothers

IV. Lifestyle Habits

31. Have you ever smoked a total of 100 cigarettes or more in your lifetime? _____ 1) Yes
2) No

If Yes:

31.1 How old were you when you first started smoking cigarettes? Age Started

31.2 Have you smoked in the last 6 months? _____ 1) Yes
_____ 2) No

If No: At what age did you stop? Age Stopped

31.3 How many cigarettes do you (or did you in the past) usually smoke?

_____ # of cigarettes

- _____ 1) Per day
 _____ 2) Per week
 _____ 3) Per month

32. How would you describe your current pattern of drinking alcohol?

- _____ 1) Do not drink at all
 _____ 2) Have an occasional drink (less than 1 per week)
 _____ 3) Usually have about 1 drink per week.
 _____ 4) Usually have about 2-6 drinks per week.
 _____ 5) Usually have about 7 drinks per week.
 _____ 6) Usually have about 8-14 drinks per week.
 _____ 7) Usually have about 15-21 drinks per week.
 _____ 8) Usually have more than 21 drinks per week.

33. How does your pattern of drinking now compare to your previous patterns of drinking alcohol?

- _____ 1) No change
 _____ 2) Drink somewhat more now than previously
 _____ 3) Drink a lot more now than previously
 _____ 4) Drink somewhat less now than previously
 _____ 5) Drink a lot less now than previously
 _____ 6) Drink more now than you drank at some times in the past and less now than you drank at other times in the past.

34. When you drink (or drank in the past), how often do you (did you) pick each of the following types of alcoholic beverages?

(Check one for each type of alcoholic beverage). *If you never drank skip to Q36*

Type	1) Never	2) Once in awhile	3) Sometimes	4) Most of the time	5) Always
A. Wine	1) _____	2) _____	3) _____	4) _____	5) _____
B. Beer	1) _____	2) _____	3) _____	4) _____	5) _____
C. Liquor	1) _____	2) _____	3) _____	4) _____	5) _____

35. On how many of the last 14 days did you have a beer, a glass of wine, or any other alcoholic drink?

- _____ 1) None
 _____ 2) 1-3
 _____ 3) 4-6
 _____ 4) 7-9
 _____ 5) 10-12
 _____ 6) 13-14

STUDY NUMBER _____

35.1. *If 1+*: On the days that you did drink, how many drinks per day, on average, did you have?

- _____ 1) One
- _____ 2) 2
- _____ 3) 3
- _____ 4) 4
- _____ 5) 5
- _____ 6) 6-9
- _____ 7) 10 or more

V. Background Information

36. What is the highest level of formal education you have completed?

(Please check one)

- _____ 1) Less than high school
- _____ 2) High school graduate
- _____ 3) Some college/trade school
- _____ 4) College graduate
- _____ 5) Post graduate

37. Are you currently employed?

- _____ 1) Yes
- _____ 2) No

If Yes:

37.1 What is your job title _____

37.2 Please describe two primary duties of your job? _____

38. To what race/ethnic group of Americans do you belong?

- _____ 1) White or European
- _____ 2) Latino, Hispanic or Mexican
- _____ 3) Black or African-American
- _____ 4) Native American or American Indian
- _____ 5) Chinese, Japanese or Korean
- _____ 6) Filipino, Vietnamese, or South East Asian
- _____ 7) Other

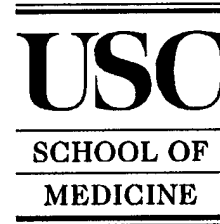
39. Please indicate your total (family) yearly household income?

- _____ 1) Below \$20,000
- _____ 2) \$20,000 - \$29,000
- _____ 3) \$30,000 - \$39,000
- _____ 4) \$40,000 - \$49,000
- _____ 5) \$50,000 - \$59,000
- _____ 6) \$60,000 or more

Please continue and complete the purple Diet Assessment by filling in the circles with a #2 pencil. Thank you.

APPENDIX 3
Letter to Participants

USC/Norris Comprehensive Cancer Center
University of Southern California
Department of Preventive Medicine
1441 Eastlake Ave, Mail Stop 44
Los Angeles, California 90033-0800



September 29, 1998

«Title»
«FirstName» «LastName»
«Address1»
«City», «State» «PostalCode»

Dear «FirstName»,

Thank you very much for agreeing to participate in this study regarding the effect of activities and other factors on estrogen levels in twins. In this box, we have enclosed all the information and supplies you will need for this study including:

- 1) Informed consent for you to sign that lists all the parts of the study that you are asked to participate in (including completion of questionnaires and collection of saliva).
- 2) Saliva donation form for you to sign
- 3) General questionnaire
- 4) Diet Assessment (purple form) with pencil
- 5) Daily physical activity logs (40 copies)
- 6) Saliva collection kit
- 7) Saliva daily log form
- 8) Carefree gum, pen, and instructions for collection of saliva
- 9) Mailing materials to mail back the informed consent, saliva donation form, General Questionnaire, Diet Assessment, and daily physical activity logs to us.
- 10) Mailing materials to mail the saliva log form and saliva collection tubes to the laboratory at Harvard University.

First of all, please read and sign the informed consent and the saliva donation form and complete the General Questionnaire and Diet Assessment. On the first day of your next menstrual period, please call us and leave a message at our Twin Study toll free 800 number (800- 421-9631). Ashley Gallagher will be the person you may speak with or you may just leave a message with your name and phone number, and Gayle Alis, our Research Assistant, will call you back.

On the next morning after your period starts, begin collecting the saliva samples. We will be measuring estrogen levels from these saliva samples and special care must be taken when collecting the samples to be sure that the hormone measurements are accurate. The procedure is to collect the saliva before you eat anything (or wait at least 15 minutes after eating (or

Ann Hamilton, Ph.D.
Assistant Professor
Preventive Medicine

323-865-0434
FAX: 323-865-0141
email: ahamilt@hsc.usc.edu

smoking)). Also, please do not brush your teeth prior to collection of the sample because brushing may cause your gums to bleed and this can throw off the measurement of the hormone levels. We are asking you to collect saliva every morning from the day this period starts until the first day of your next menstrual flow (about 30 days).

The tubes are marked with your study number; however we ask you to write the date and time of collection on each tube when you collect the sample and also to put your initials on it. On the days when you have menstrual flow, please mark those tubes with an 'M'. Please also record this information on the 'Saliva Daily Log Form'. Chewing the gum we have provided will help to stimulate saliva flow. Please fill the tube to the bottom of the tape. Please do not use any other brand of gum.

The tubes should be kept in a safe place away from children and pets; however they do not require refrigeration or any special conditions. When your time of collection is completed (i.e. your next period has started), please make sure the tubes are tightly capped and mail the tubes to the laboratory at Harvard in the packaging provided. The postage and address label are already on the box; thus all you need to do is to put the container with the tubes in the box, stuff some of the bubble wrap (or a little paper) around the container, seal the box with the tape provided, and put it in the mail.

On the days when you are collecting the saliva samples, we are also asking you to fill out a daily physical activity log at the end of each day. When the sample collection is completed, please insert the informed consent, the saliva donation form, the General Questionnaire, the Diet Assessment, and the daily physical activity logs into the mailing envelope provided (with prepaid postage and address already on it) and put it in the mail to us.

If, at any time, you have any questions or problems please call us on our toll free Twin Study number (800-421-9631) and leave a message. You may also call either of us and leave a message, or we will call you right back to minimize any phone charges to you. Gayle Alis, our Research Assistant, will be calling you to see how things are going.

Thank you again for your willingness to participate in this very important study. We will keep you informed about the results.

Sincerely,

Ann S. Hamilton, Ph.D.
Co-Principal Investigator
323-865-0434

Lisa Shames, Ph.D.
Co-Principal Investigator
323-865-0422

APPENDIX 4
Informed Consent

Dr. Ann Hamilton
Dr. Lisa Shames
(323) 865-0434
NorrisCancer Center/USC School of Medicine
1441 Eastlake Ave. MS #44
Los Angeles, CA 90033

Initials _____

**An Innovative Assessment of Endogenous Estrogen Activity in Persons with
Different Habits of Exercise**

Participant Informed Consent

PURPOSE: This study is being conducted in an effort to learn more about the effects of physical activity on ovarian hormones in premenopausal women.

PROCEDURE: Your participation in this study involves three aspects. First, you will be asked to complete a survey regarding your exercise habits, dietary intake and personal medical history. Second, you will be asked to collect 5ml's (approximately 5 teaspoons) of saliva (in a plastic tube) over an entire menstrual cycle (or approximately 30 days) by spitting into a tube. The saliva collection should be done one time per day when you first wake up in the morning. Detailed information on how the saliva samples are to be collected are included in the study packet on a form entitled 'Instruction for Participants'. The tube is pre-treated with a small amount of a bactericidal agent called sodium azide. The sodium azide is at the bottom of each tube and is visible as a small dried residue which adheres firmly to the base of each tube. Please keep tubes out of the reach of small children since this agent is toxic when ingested. Lastly, you will be asked to keep a daily log of your physical activities (we will supply you with these forms) over one menstrual cycle.

The entire study will performed over one menstrual cycles (or about 30 days) and will be done in the convenience of your home. We will mail you a study packet complete with instructions, collection tubes, and surveys. The packet should be mailed to the laboratory upon completion of your participation in the study (we will pre-pay postage).

RISKS: There are no conceivable health risks associated with the collection of one's own saliva. However, care should be exercised when storing tubes in a home with small children, since the bactericidal agent adhering to the bottom of each tube (which keeps the specimens fresh), can be toxic if swallowed.

BENEFITS: There are no direct benefits to you. There is, however, the indirect benefit of knowing that you are contributing to a study that may help us better understand how physical activity affects women's health.

ALTERNATIVES: It is understood that you may choose not to participate in this study and that your decision will not in any manner affect your medical care. This study is designed to assess hormone levels over an entire menstrual cycle and there are no other convenient non-invasive

Initials _____

alternatives to this method of sample collection. You are free to withdraw your consent and discontinue participation at any time.

CONFIDENTIALITY: The confidentiality of any information obtained through this study will be maintained by the investigators. Any information which personally identifies you will not be released or disclosed without your written consent except as specifically required by law. Representatives of the U.S. Army Medical Research and Materiel Command are eligible to review research records as part of their responsibility to protect human subjects in research.

OFFER TO ANSWER QUESTIONS: It is understood that if you have any questions, comments or concerns about the study or the informed consent process, you should contact the Principal Investigators; Dr. Ann Hamilton or Dr. Lisa Shames with the Department of Preventive Medicine, 1441 Eastlake Ave., Los Angeles, CA 90033 ((323) 865-0434). If you have any questions regarding your rights as a study participant, you may contact the Institutional Review Board Office at (213) 223-2340. You will be given a copy of this form to keep.

COERCION AND WITHDRAWAL STATEMENT: Your decision whether or not to participate will not interfere with your medical treatment at this university. You are free to withdraw your consent and to discontinue participation at any time. You may decline to answer any survey questions you wish to.

INJURY STATEMENT: If you should require medical treatment as a result of an injury arising from your participation in this study, the financial responsibility for such treatment will be yours.

NEW INFORMATION: Any new information that is developed during the course of this research which may be related to your willingness to continue or discontinue participation in this study will be provided to you.

CALIFORNIA LAW REQUIRES THAT YOU MUST BE INFORMED ABOUT:

1. The nature and purpose of the study.
2. The procedures in the study.
3. Discomforts and risks to be expected from the study.
4. Benefits to be expected from the study.
5. Alternative procedures that might be helpful and their risks and benefits.
6. Availability of medical treatment should complications occur.
7. The opportunity to ask questions about the study or the procedure.
8. The opportunity to withdraw at any time.
9. A copy of the written consent from the study.
10. The opportunity to consent freely to the study without coercion.
11. Statement regarding liability for research-related injury (if applicable).

AGREEMENT: Your signature indicates your decision to participate in this study having read the information provided above.

Signature of Volunteer

Date

Signature of Investigator

Date

Date: July 27, 1998

Form Valid For Enrollment From AUG 15 1998 To AUG 14 1999 Institutional Review Board
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APPENDIX 5
Saliva Donation Form

**UNIVERSITY OF SOUTHERN CALIFORNIA
CONSENT FORM FOR STUDY ENTITLED:
AN INNOVATIVE ASSESSMENT OF ENDOGENOUS ESTROGEN ACTIVITY IN
PERSONS WITH DIFFERENT HABITS OF EXERCISE**

I, _____, voluntarily and freely donate my saliva sample to USC for analysis, and thereby relinquish all right, title, and interest to this sample.

Participant's signature

Participant's name (printed)

Date of signature

APPENDIX 6

Physical Activity Daily Log

Please fill out one copy of this form each day
during saliva collection period

Today's Date: ____/____/____
Month Day Year

Daily Log: Physical Activity Profile:

1. Please list any sport or recreation you have participated in during the past 24 Hours. Please include only the time you were physically active (i.e. actual exercise time while jogging, bicycling, swimming, brisk walking, gardening, carpentry, calisthenics, etc).

NAME OF SPORT, RECREATION OR OTHER PHYSICAL ACTIVITY	Number of TIMES you participated in the activity today	Average TIME spent per episode doing the activity	Total TIME of participation in activity today
		HOURS / MINUTES	
1.		/	
2.		/	
3.		/	
4.		/	
5.		/	

2. Approximately how many flights (not numbers) of stairs did you climb up TODAY?

Flights

3. Approximately how many city blocks or their equivalent did you walk TODAY?

Blocks

4. How much time did you spend on the following activities TODAY. Total should add up to 24 hours.

TYPE OF ACTIVITY	HOURS TODAY
a. Vigorous activity (e.g., digging in the garden, strenuous sports, jogging, chopping wood, sustained swimming, brisk walking, heavy carpentry, bicycling on hills)	
b. Moderate activity (e.g., housework, light sports, regular walking, golf, yard work, lawn mowing, painting, repairing,, light carpentry, dancing, bicycling on level ground)	
c. Light activity (e.g., office work, driving a car, strolling, personal care, standing with little motion)	
d. Sitting activity (eating, reading, desk work, watching TV, listening to radio)	
e. Sleeping or reclining	

Total=24 hours

6. Did you engage in regular activity akin to brisk walking, jogging, bicycling, etc. long enough to work up a sweat, get your heart thumping, or get out of breath TODAY?

____ 1) Yes

____ 2) No

7. What was your usual pace of walking TODAY? (Please check one)

____ 1) Casual or strolling (<2 mph)

____ 2) Average or normal (2 to <3 mph)

____ 3) Fairly brisk (3 to <4 mph)

____ 4) Brisk or striding (4 mph or faster)

APPENDIX 7

Willett Diet Assessment

5	6	7	8	9	0	1	2	3	4	5	6	7	8	9
5	6	7	8	9	0	1	2	3	4	5	6	7	8	9
5	6	7	8	9	0	1	2	3	4	5	6	7	8	9
5	6	7	8	9	0	1	2	3	4	5	6	7	8	9
5	6	7	8	9	0	1	2	3	4	5	6	7	8	9
5	6	7	8	9	0	1	2	3	4	5	6	7	8	9
5	6	7	8	9	0	1	2	3	4	5	6	7	8	9
5	6	7	8	9	0	1	2	3	4	5	6	7	8	9

ID:

1. Do you currently take multiple vitamins? (Please report individual vitamins under question 2.)

☐ No ☐ Yes → If yes, a) How many do you take per week? ☐ 2 or less ☐ 3-5 ☐ 6-9 ☐ 10 or more

b) What specific brand do you usually use?

Specify exact brand and type

2. Not counting multiple vitamins, do you take any of the following preparations:

a) Vitamin A?

☐ No ☐ Yes, seasonal only ☐ Yes, most months } If Yes, How many years? ☐ 0-1 yr. ☐ 2-4 yrs. ☐ 5-9 yrs. ☐ 10+ yrs. ☐ Don't know
What dose per day? ☐ Less than 8,000 IU ☐ 8,000 to 12,000 IU ☐ 13,000 to 22,000 IU ☐ 23,000 IU or more ☐ Don't know

b) Vitamin C?

☐ No ☐ Yes, seasonal only ☐ Yes, most months } If Yes, How many years? ☐ 0-1 yr. ☐ 2-4 yrs. ☐ 5-9 yrs. ☐ 10+ yrs. ☐ Don't know
What dose per day? ☐ Less than 400 mg. ☐ 400 to 700 mg. ☐ 750 to 1250 mg. ☐ 1300 mg. or more ☐ Don't know

c) Vitamin B₆?

☐ No ☐ Yes → If yes, How many years? ☐ 0-1 yr. ☐ 2-4 yrs. ☐ 5-9 yrs. ☐ 10+ yrs. ☐ Don't know
What dose per day? ☐ Less than 10 mg. ☐ 10 to 39 mg. ☐ 40 to 79 mg. ☐ 80 mg. or more ☐ Don't know

d) Vitamin E?

☐ No ☐ Yes → If yes, How many years? ☐ 0-1 yr. ☐ 2-4 yrs. ☐ 5-9 yrs. ☐ 10+ yrs. ☐ Don't know
What dose per day? ☐ Less than 100 IU ☐ 100 to 250 IU ☐ 300 to 500 IU ☐ 600 IU or more ☐ Don't know

e) Selenium?

☐ No ☐ Yes → If yes, How many years? ☐ 0-1 yr. ☐ 2-4 yrs. ☐ 5-9 yrs. ☐ 10+ yrs. ☐ Don't know
What dose per day? ☐ Less than 80 mcg. ☐ 80 to 130 mcg. ☐ 140 to 250 mcg. ☐ 260 mcg. or more ☐ Don't know

f) Iron?

☐ No ☐ Yes → If yes, How many years? ☐ 0-1 yr. ☐ 2-4 yrs. ☐ 5-9 yrs. ☐ 10+ yrs. ☐ Don't know
What dose per day? ☐ Less than 51 mg. ☐ 51 to 200 mg. ☐ 201 to 400 mg. ☐ 401 mg. or more ☐ Don't know

g) Zinc?

☐ No ☐ Yes → If yes, How many years? ☐ 0-1 yr. ☐ 2-4 yrs. ☐ 5-9 yrs. ☐ 10+ yrs. ☐ Don't know
What dose per day? ☐ Less than 25 mg. ☐ 25 to 74 mg. ☐ 75 to 100 mg. ☐ 101 mg. or more ☐ Don't know

h) Calcium?

(Include Calcium in Dolomite.)

☐ No ☐ Yes → If yes, How many years? ☐ 0-1 yr. ☐ 2-4 yrs. ☐ 5-9 yrs. ☐ 10+ yrs. ☐ Don't know
What dose per day? ☐ Less than 400 mg. ☐ 400 to 900 mg. ☐ 901 to 1300 mg. ☐ 1301 mg. or more ☐ Don't know

i) Are there other supplements that you take on a regular basis? Please mark if yes:

☐ Folic acid ☐ Cod liver Oil ☐ Iodine ☐ Beta-Carotene ☐ Other (please specify):
☐ Vitamin D ☐ Copper ☐ Brewer's Yeast
☐ B-Complex Vitamins ☐ Omega-3 Fatty-acids ☐ Magnesium

3. For each food listed, fill in the circle indicating how often on average you have used the amount specified during the past year.

		AVERAGE USE LAST YEAR									
		Never, or less than once per month	1-3 per mo.	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4-5 per day	6+ per day	
DAIRY FOODS											
Skim or low fat milk (8 oz. glass)		<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Whole milk (8 oz. glass)		<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Cream, e.g. coffee, whipped (Tbs)		<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sour cream (Tbs)		<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Non-dairy coffee whitener (tsp.)		<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sherbet or ice milk (1/2 cup)		<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Ice cream (1/2 cup)		<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Yogurt (1 cup)		<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Cottage or ricotta cheese (1/2 cup)		<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Cream cheese (1 oz.)		<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Other cheese, e.g. American, cheddar, etc., plain or as part of a dish (1 slice or 1 oz. serving)		<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Margarine (pat), added to food or bread; exclude use in cooking		<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Butter (pat), added to food or bread; exclude use in cooking		<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

3. (Continued) Please fill in your average use, during the past year, of each specified food.

Please try to average your seasonal use of foods over the entire year. For example, if a food such as cantaloupe is eaten 4 times a week during the approximate 3 months that it is in season, then the average use would be once per week.

	Never, or less than once per month	1-3 per mo.	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4-5 per day	6+ per day	
FRUITS										
Raisins (1 oz. or small pack) or grapes	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Prunes (1/2 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Bananas (1)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Cantaloupe (1/4 melon)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Watermelon (1 slice)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Fresh apples or pears (1)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Apple juice or cider (small glass)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Oranges (1)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Orange juice (small glass)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Grapefruit (1/2)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Grapefruit juice (small glass)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Other fruit juices (small glass)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Strawberries, fresh, frozen or canned (1/2 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Blueberries, fresh, frozen or canned (1/2 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Peaches, apricots or plums (1 fresh, or 1/2 cup canned)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

	Never, or less than once per month	1-3 per mo.	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4-5 per day	6+ per day	
VEGETABLES										
Tomatoes (1)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Tomato juice (small glass)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Tomato sauce (1/2 cup) e.g. spaghetti sauce	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Red chili sauce (1 Tbs)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Tofu or soybeans (3-4 oz.)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
String beans (1/2 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Broccoli (1/2 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Cabbage or cole slaw (1/2 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Cauliflower (1/2 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Brussels sprouts (1/2 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Carrots, raw (1/2 carrot or 2-4 sticks)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Carrots, cooked (1/2 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Corn (1 ear or 1/2 cup frozen or canned)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Peas, or lima beans (1/2 cup fresh, frozen, canned)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Mixed vegetables (1/2 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Beans or lentils, baked or dried (1/2 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Yellow (winter) squash (1/2 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Eggplant, zucchini, or other summer squash (1/2 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Yams or sweet potatoes (1/2 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Spinach, cooked (1/2 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Spinach, raw as in salad	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Kale, mustard or chard greens (1/2 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Iceberg or head lettuce (serving)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Romaine or leaf lettuce (serving)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Celery (4" stick)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Beets (1/2 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Alfalfa sprouts (1/2 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Garlic, fresh or powdered (1 clove or shake)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

	Never, or less than once per month	1-3 per mo.	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4-5 per day	6+ per day	
EGGS, MEAT, ETC.										
Eggs (1)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Chicken or turkey, with skin (4-6 oz.)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Chicken or turkey, without skin (4-6 oz.)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Bacon (2 slices)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Hot dogs (1)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

3. (Continued) Please fill in your average use,
during the past year, of each specified food.

	Never, or less than once per month	1-3 per mo.	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4-5 per day	6+ per day
MEATS (CONTINUED)									
Processed meats, e.g. sausage, salami, bologna, etc. (piece or slice)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Liver (3-4 oz.)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Hamburger (1 patty)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Beef, pork, or lamb as a sandwich or mixed dish, e.g. stew, casserole, lasagne, etc.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Beef, pork, or lamb as a main dish, e.g. steak, roast, ham, etc. (4-6 oz.)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Canned tuna fish (3-4 oz.)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Dark meat fish, e.g. mackerel, salmon, sardines, bluefish, swordfish (3-5 oz.)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Other fish (3-5 oz.)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Shrimp, lobster, scallops as a main dish	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

	Never, or less than once per month	1-3 per mo.	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4-5 per day	6+ per day
BREADS, CEREALS, STARCHES									
Cold breakfast cereal (1 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Cooked oatmeal (1 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Other cooked breakfast cereal (1 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
White bread (slice), including pita bread	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Dark bread (slice)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
English muffins, bagels, or rolls (1)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Muffins or biscuits (1)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Brown rice (1 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
White rice (1 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Pasta, e.g. spaghetti, noodles, etc. (1 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Other grains, e.g. bulgar, kasha, couscous, etc. (1 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Pancakes or waffles (serving)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
French fried potatoes (4 oz.)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Potatoes, baked, boiled (1) or mashed (1 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Potato chips or corn chips (small bag or 1 oz.)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Crackers, Triskets, Wheat Thins (1)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Pizza (2 slices)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

			Never, or less than once per month	1-3 per mo.	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4-5 per day	6+ per day
CARBONATED BEVERAGES		BEVERAGES									
Consider the serving size as 1 glass, bottle or can for these carbonated beverages.	Low Calorie (sugar-free) types	Low calorie cola, e.g. Tab with caffeine	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
		Low calorie caffeine-free cola, e.g. Pepsi Free	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
		Other low calorie carbonated beverage, e.g. Fresca, Diet 7-Up, diet ginger ale	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Regular types (not sugar-free)	Coke, Pepsi, or other cola with sugar	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
		Caffeine Free Coke, Pepsi, or other cola with sugar	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
		Other carbonated beverage with sugar, e.g. 7-Up, ginger ale	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
OTHER BEVERAGES		Hawaiian Punch, lemonade, or other non-carbonated fruit drinks (1 glass, bottle, can)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
		Decaffeinated coffee (1 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
		Coffee (1 cup)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
		Tea (1 cup), not herbal teas	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
		Beer (1 glass, bottle, can)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
		Red wine (4 oz. glass)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
		White wine (4 oz. glass)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
		Liquor, e.g. whiskey, gin, etc. (1 drink or shot)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

3. (Continued) Please fill in your average use during the past year, of each specified food.

[illegible]

☐ Remove all visible fat ☐ Remove small part of fat
☐ Remove majority ☐ Remove none
☐ (Don't eat meat)

☐ Real butter ☐ Vegetable oil ☐ Lard
☐ Margarine ☐ Vegetable shortening

☐ Real butter ☐ Vegetable oil ☐ Lard
☐ Margarine ☐ Vegetable shortening

☐ None ☐ Stick ☐ Tub ☐ Spread
☐ Low-calorie stick ☐ Low-calorie tub

☐ Daily ☐ 4-6 times per week

☐ 1-3 times per week ☐ Less than once a week

☐ Daily ☐ 4-6 times per week

☐ 1-3 times per week ☐ Less than once a week

<p>12. What kind of cold breakfast cereal do you usually use? →</p>	<p>Specify type and brand</p>
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(Do not include dry spices and do not list something that has been listed in the previous sections.)

Other foods that you usually use at least once per week	Usual serving size	Servings per week
(a)		
(b)		
(c)		
(d)		

APPENDIX 8
Saliva Collection Instructions

COLLECTING SALIVA SAMPLES

Participation in this part of the study involves collecting a **morning** saliva sample **every day** over the course of one menstrual cycle. While it is not necessary that collection be done at exactly the same time each day, we hope that you will find a way to fit sample collection into your daily routine, so that it will constitute the least possible burden to you.

- **Begin collecting on the first day of your menstrual cycle** (that is, when your menstrual bleeding starts, begin your collection the following morning). **Collect a sample every day until the start of the next menstrual period. Please collect in the morning.**
- Do not eat, drink, chew or smoke anything for *at least* 15 minutes before collecting a sample. Food (for example, coffee, egg) contamination can significantly distort hormone readings.
- Make sure your mouth is not bleeding for any reason before collecting a saliva sample, as even a tiny amount of blood can throw off the measurement of hormone levels. If your gums are bleeding, try rinsing your mouth with cold water, waiting several minutes, and trying again.
- Do not brush your teeth immediately before collecting a sample, since brushing can cause your gums to bleed.
- If you do happen to miss a day, just continue the collection the next morning.

Instructions for saliva collection:

- **Using the indelible pen provided**, mark a tube (on the tape) with your **initials** and the **time** and the **date** of collection. Also, mark the sample tubes collected on the days of menstrual flow with an "M".
- Chew a piece of the gum provided for a few seconds to stimulate saliva flow (you may prefer to use only half a piece of gum). Then carefully fill the tube **to the bottom of the tape** with saliva. If you have difficulty getting the saliva into the tube on the first couple of tries, you may find it useful to look in the mirror while you are collecting. Please use **only** the gum provided, as this has been screened for use with our assays. It is not necessary to remove the gum from your mouth before collecting the sample. If you get a lot of bubbles in the tube, get rid of them by tapping the bottom of the tube a few times sharply on a hard surface, then continue to fill the tube with saliva to the tape.
- Cap the tube securely.
- Keep the collected samples in the container provided. The tubes have been pretreated with a special preservative and do not require refrigeration or freezing. The preservative used, sodium azide, is toxic if ingested. It is coated on the inner surface of the bottom of the tubes and does not represent a health hazard under normal collection conditions. **However, please store the tubes out of the reach of small children and pets and do not re-ingest any saliva from the tube following collection.**
- When your sample collection is completed make sure each tube is tightly capped, and then mail the tubes back to us in the container and the box the tubes were shipped in, using the Priority Mail stamp and address label provided.

APPENDIX 9
Saliva Collection Log Form

Saliva Collection Log Form

Twin Name: _____

ID Number: _____

*Please enter the dates and times saliva was collected and days of menstrual flow.
Please send this sheet to laboratory in the box with the tubes. Thank you.*

Date of Collection	Time of Collection	Menstrual Flow? Yes/No
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.		
12.		
13.		
14.		
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